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PRINCIPAL INVESTIGATOR: Wadie F. Bahou, M.D.

CONTRACTING ORGANIZATION: State University of New York
Stony Brook, NY 11794-3362

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14. ABSTRACT

During the last FY of the award, the Cancer Institute of Long Island benefited from CDMRP funding in a manner consistent with the proposed activities of the award. In the area of core instrumentation acquisition, an Olympus upright microscope has been added and integrated in to the previously funded two –photon system. The new instrument is essential for in vivo imaging for mice and rats. The previously funded Zeiss TIRF Microscope system is now operational. A new, state-of-the-art VisualSonics small animal ultrasound has been brought online to support mouse tumor models. The University has endeavored to create a Mouse Metabolic Phenotyping Core to support cancer research activities for cancer researchers. Consistent with the funding for this initiative were the granting of multi-year RSU packages, or Research Support Unit. RSU's are a mechanism to ensure necessary support for junior faculty and work as enhancements to enable the successful establishment of their laboratories. The final year of funding for Dr. Mirjana Maletic-Savatic, M.D., Ph.D., Assistant Professor of Neurology (new RSU) was provided via this mechanism. Her laboratory is now fully established and operational.

15. SUBJECT TERMS
In vivo imaging, Mouse Phenotyping, Small Animal Ultrasound, TIRF imaging

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Introduction:

The State University of New York at Stony Brook, School of Medicine continues to develop its infrastructure to support a Comprehensive Cancer Center in central Long Island, New York. Achievements vital to this year of CDMRP funding are summarized below. CDMRP funding focused in cancer research infrastructure has greatly assisted the efforts of the School of Medicine by enabling our ability to provide a foundation for aspiring young scientists. Dr. Maletic-Savatic received year two base support from this mechanism to further develop her research program. CDMRP funds allocated towards core technologies have enabled the School of Medicine to secure and bring on-line a new state-of-the-art Total Internal Reflection Fluorescence Microscope. Additionally the CDMRP funded two-photon confocal system was up graded to include an Olympus upright in vivo scope. This system is two-photon capable and is used for live animal microsurgeries under two photon conditions. The instrument is located in an ISO Class 7 clean room to help ensure the most sterile conditions possible. A VisualSonics Small Animal Ultrasound was acquired to enable high throughput cancer marker screens in mice and rats. The School of Medicine has commenced the establishment of a well staffed core for mouse metaballomic phenotyping research. This core is available to all cancer researchers in the University. All instrumentation is centrally situated, and supported by ancillary equipment made available by the School of Medicine via other funding sources (not CDMRP).

Body:

III) Dr. Mirjana Maletic-Savatic, MD-PhD, Assistant Professor Neurology:

Human Neural Stem Cells – In Vivo Models for Cerebral Carcinoma

The study of human neural stem cells (NSC) in vivo has been hindered by the absence of well-defined markers that would distinguish them from other neural cell types, such as astrocytes, oligodendrocytes and neurons. We analyzed mouse derived cultured hippocampal neurons, glia, and NSC in order to identify spectroscopic signatures for each individual cell type. One dimensional 1H-NMR spectra were collected using a Bruker Avance 700 NMR spectrometer, working at a hydrogen resonance frequency of 700.13 MHz. Over the past year, our preliminary data suggest the presence of specific spectroscopic profiles for each individual cell type studied, thus providing for identification and quantification of NSC. We have detected the NSC-specific spectroscopic signatures in the brain extracts as well. In collaboration with Dr. Djuric, Department of Engineering, we have developed more sophisticated data processing algorithms for extracting the NSC-specific peak from data with low resolution. More recently, we applied our results to human brain imaging and were able to extract a NSC peak from the hippocampus and not cortex, which corresponds to animal data. Our results were presented at the Keystone Symposia on stem cells. A manuscript is in preparation, also. The plan for the next year is to start characterization of the metabolite which gives the NSC-specific spectra and to continue with the human brain imaging. Our results may ultimately lay the foundation for future studies of NSC fate and function in the living human brain, with immediate consequence for the clinical management of a spectrum of neurological diseases such as cerebral carcinoma, and multiple sclerosis.

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Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724, USA.

Cancer Imagining Core Research Support

To broadly support the research of the five-thematic integrated cancer research programs several new core imaging platforms are moving ahead under various states of maturity. These include (A) In Vivo Two-Photon Imaging System- 2003, (B) Small Animal Ultrasound, and (C) Total Internal Reflection Fluorescence (TIRF) Imaging System- 2005.

A) The two-photon in vivo microscope is a custom made tool used to dissect processes in the living tissue using fluorescent protein imaging. The microscope has two stages. One stage is used to perform electrophysiology and imaging of cellular and subcellular mechanisms in real time, in acute brain slices and in organotypic slice cultures. We use this methodology to observe and manipulate the interactions between neural stem cells and the microglia, the scavengers of the nervous tissue, in order to better understand the mechanisms that determine the survival of endogenous and transplanted neural stem cells. In addition, we use slices to investigate the mechanisms that lead to formation of new neurons, and we monitor this process of neurogenesis in real time. The second stage is used for live animal imaging. We use transgenic mice in which certain cell types can be visualized due to the expression of fluorescent proteins. Therefore, the cells of interest are readily seen, up to 400 micrometer deep. We are again interested in processes that lead to incorporation of transplanted neural stem cells into the normal circuitry of the brain, as this is one of the most promising therapies for several neurological disorders, such as brain tumors and strokes. Therefore, the availability of this microscope enables us to investigate processes and mechanisms that we have never been able to investigate before, putting Stony Brook at the cutting edge of science. The software & hardware will be brought online by the Office of Scientific Affairs, SOM Bioinformatics Service. The two 1.0 FTE programmers of this unit provide IT support to all of the core technologies that are assigned to the Office of Scientific Affairs. Competitive intramural matching support from the School of Medicine and Vice-

President for Research will offset partial 1.0 FTE Technician costs, and service contract costs.

B) The VisualSonics Vevo small animal ultrasound imager was acquired this year and sited in the University Laboratory Animal Facility where it receives dedicated support from a 1.0 FTE Ultrasound Technician trained in rodent anatomy. The instrument is used to phenotype a variety of cancer models, to monitor disease progression, and to quantify and inject cancer vaccines into specific organs. In particular, specially developed transgenic mouse models for 3 phenotypically distinct types of human bladder cancer are being imaged by the CS Lee Laboratory, Dept. of Urology. Transitional cell cancer (TCC) models utilizing SV40 to inactivate tumor suppressor genes produce two different models. Low copy insertions result in *carcinoma in situ* and high copy insertions in high grade invasive TCC. A third model utilizing *ras* inserted transgenes produce low grade papillary tumors in the mice. The ultrasound instrument will allow non-invasive measurement of tumor volume, shape and vascularity. We plan to study novel bladder cancer vaccines made with recombinant BCG, developed in collaboration with Michael O'Donnell at University of Iowa: rBCG-IFN-g, rBCG-IL-2, rBCG-TNF and rBCG-GM-CSF. The vaccines can be instilled under ultrasound guidance into the bladder of the affected mice and the tumor regressions are followed in real-time. The instrument also allows guided removal of urine samples from the mice in order to study cytokines and tumor markers in those samples. Such markers are analysed in the Mouse Metabolic Phenotyping Core. Since ultrasound imaging is used to monitor human bladder cancer, the availability of this instrument will enable rapid pre-clinical studies for bladder cancer and anti-cancer vaccines that can lead directly into human clinical trials.

C) The TIRF System described below is now on-line:
Total Internal Reflection Fluorescence (TIRF) Imaging System. The basis for TIRF is the refractive behavior of light when making the transition from an optically denser to an optically less dense medium. The analysis of images obtained with conventional wide-field fluorescence excitation is often complicated by background fluorescence emitted in out-of-focus planes. The signals from these regions radiate into the depth-of-field range and superimpose themselves upon the desired image information. The effect is due to the comparatively low Z resolution achievable with this illuminating technique. By contrast, fluorescence excited by total internal reflection (TIRF) yields excellent Z resolution, typically around 200 nm or better. This is clearly illustrated by the following example. Fluorescent beads were mixed with distilled water and put on a specimen slide, with a cover slip on top. In a fresh preparation, the beads are constantly moving between the slide and the cover slip due to Brownian motion. After a short time, however, the first beads start to deposit on the slide and on the cover slip.

In conventional fluorescence microscopy, images also show beads from above the focal plane, where as TIRF microscopy offers information exclusively from the evanescent field. In fluorescence microscopy, beads approaching the cover slip become visible long before they reach the focal plane, whereas TIRF microscopy produces a fluorescence signal only when the beads have entered the narrow band of the evanescent field. The signals suddenly vanish when the beads leave that field. The advantages of TIRF microscopy are obvious: no superimposed background fluorescence, and higher resolution, resulting in better contrast and high-fidelity detail rendition. Total reflection occurs at interface such as between glass and water. Therefore, TIRF is a useful tool for

studying the reactions of individual molecules or objects at surfaces. A typical application in molecular cell biology is the fusion of vesicles with the cell membrane.

The TIRF is available to cancer researchers at Stony Brook. This technique is considered to provide an excellent bridge between the two previous confocal techniques which have been funded via the CDMRP. Left alone the TIRF technology is a powerful tool of discovery for cellular activity at the membrane level. The ability to witness the events related to cancer cell membrane breaching by novel therapeutics is obviously important to new drug development. Due to the fact that TIRF is an epi-fluorescence based imaging technique, clinical cancer researchers acclimate easily to this instrument.

Cancer Metaballomics Assay Core

The focus of the integrative Cancer Mouse Meatbollomics Assay Core is to determine collateral endocrine disruption due to cancer therapeutics. Among the tasks will be monitor and develop novel phonotypical mice that exhibit a variety of adverse responses to cancer therapeutics. Using a host of complicated assays the core staff (2.0 FTE's) will develop a reference set of downstream complications that will challenge pharmacological intervention. There is little understanding of the field of metaballomics within the domain of cancer. Stony Brook will seek to leverage its scientific expertise in diabetes to begin to tease out the complications of metabolic damage in cancer. The core will be located within our Division of Laboratory Animal Resources facility in the Health Sciences Center.

Key Research Accomplishments:

The CDMRP funding awarded to Stony Brook via this mechanism is directed towards providing infrastructure support to better serve the needs of the faculty of the Cancer Institute of Long Island, and cancer researchers throughout the campus. Accomplishments for this reporting period include:

1) Instrumentation-Carl Zeiss Multi-Photon Confocal Microscope on-line and participating in more than 43 active cancer projects *.
2) Instrumentation- Continued support for the ISO 7 clean room for the Multi-Photon Microscope *.
3) Instrumentation- Renewal of the PhD level microcopist to operate the Multi-Photon Microscope *.
4) Instrumentation- Continued support for the high-level image analysis center to support the Multi-Photon Confocal Microscope users **.
5) Instrumentation- On going of FEI Philips Digital Transmission Electron Microscope for cancer imaging **.
6) Instrumentation- Continued support of an ISO 7 clean room for the Digital Transmission Electron Microscope *.
7) Instrumentation- On going suport and operation of an ABI 3730 High-Throughput Genetic Analyzer *.
8) Instrumentation- Continued support for the BioRad and ABI Research Real-Time PCR instruments **.
9) Instrumentation- Development of protocols to further enhance the through-put capabilities in MALDI-ToF for Cancer Proteomics **.
10) Instrumentation- Newly developed Maual Tryptic Digestion Protocols service made available for faculty**.

11)Faculty Development- Providing start-up funds enhancement to Dr. Adler via a mentored intramural program *.
12)Acquisition of a Small Animal UltraSound Instrument and identification of a dedicated technician.*
13)Faculty Development- Providing start-up funds enhancement to Dr. Maletic-Savatic via a mentored intramural program*.
14)More than 20 key publications produced by the cancer research faculty in the School of Medicine. A fully annotated citation list is available for review in the appendix of this report **.
15) Sixteen cancer research intramural pilot and feasibility awards issued via the School of Medicine Targeted Research Opportunities Program **.
16)Creation of a Cancer Chemo-Prevention Center Laboratory in the Dept. of Medicine **.

*= *Benefit derivative of CDMRP funds.*
**= *Benefit derivative of funds allocated to complement CDMRP initiative at Stony Brook.*

Reportable Outcomes:

1- Research Support Units- The RSU support provided to Dr. Maletic-Savatic have resulted in several manuscripts for peer reviewed journals.
2- Cancer Genomics Core- To date this facility has provided services that have resulted in tens of thousands of sequences and validations for samples submitted by Cancer Institute researchers. The RT PCR instruments and the Genetic Analyzer are enhancing an already robust research core before the end of CY 2006, the Affymetrix GeneChip Core, the Cancer Genomics Core and the Bioinformatics Core will integrated into a Functional Genomics Resource Center.
3- The Applied Biosystems Q-Star Pulsar I LC/MS/MS instrument acquired in year 01 of the CDMRP award has logged over 3,800 sample hours since its commissioning. Recently the School of Medicine has invested in upgrading the system to a grid less platform thus reducing downtime inefficiency.
3- Cancer Imaging Core- The Multi-Photon capabilities have been well received by the cancer research community. The instrument performs at threshold levels. To date more than 44 experiments were imaged as time-course experiments on this system. As previously stated the platform is being upgraded to include an in vivo upright two photon surgical microscope. The new linkage between the University funded Digital Transmission Electron Microscope and the Two Photon Systems is complete. They now sit as an integrated core facility.
The FCS instrument is on-line and performing as anticipated.
4- A seminar in Biacore Palsmon Resonance Technology is being coordinated throughj the Proteomics Center, and the Affymetrix Core is developing a seminar schedule for cancer researchers later this year.

Conclusions:

T the beneficial infrastructure support that the CDMRP provides to the School of Medicine enables our ability to actively support the newest scientific technologies available to cancer researchers. The new integration of the cancer cores and

development of an assay specific resource service are examples of expansion of an already robust research infrastructure. CDMRP funds continue to make this possible for Stony Brook. Working collaboratively with CDMRP in 2005-2006, we have been able to bring the newest platform instrumentation while developing state-of-the-art facilities to advance the Stony Brook cancer research community significantly

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Not applicable. CDMRP funding is targeted towards infrastructure support.

Appendicies:

12 selected publications of interest. Reprints are available upon request.

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